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Bioactive content and antioxidant capacity of some plants and fruits grown in Türkiye

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Abstract: Recently, some edible plants and fruits have undergone extensive research to identify potential health effects arising from their valuable nutritional and bioactive properties. The present study aims to investigate the phenolic compounds of nine edible plants and fruits throughout the gastrointestinal digestion model by comparing their total content and antioxidant activity. Of the species studied, *Eremurus spectabilis* and *Gundelia tournefortii* had the lowest total phenolic content, whereas *Prunus armeniaca* showed the highest value of 1026 ± 164 mg GAE/100 g. Except for *Prunus armeniaca* , the total phenolic contents of all samples were increased following gastric digestion, while the total flavonoid contents were decreased. Similarly, the DPPH scavenging activities of the *Celtis tournefortii*, *Crocus cancellatus* subsp. *damascenus*, and *Gundelia tournefortii* var. *tournefortii* samples increased remarkably after gastric digestion by 70-fold, ~11-fold, and ~4-fold, respectively. The cupric-reducing antioxidant capacity assay showed that all samples except *Prunus armeniaca*, *Morus alba*, and *Rheum ribes* had increased antioxidant activity following gastric digestion, which was observed to decrease after intestinal digestion. It can be concluded that the total phenolic content of some edible plants and fruits collected from the eastern Anatolia region of Türkiye yielded relatively valuable results, and controlled dietary intake of these plants may have the potential to show positive effects on health due to their high antioxidant activity.

Key words: phenolic compounds, antioxidant activity, bioaccessibility, bioactivity, edible plants

1. Introduction

More than 7000 different species of edible plants have been reported to be used for human consumption since ancient times (Grivetti and Ogle, 2000). A recent review of the literature shows that several edible plants have a crucial role in many regions, supplying seasonal food as well as a cultural identity to consumers (Borelli et al., 2020). Together with the current shift to plant-based sustainable diets worldwide, edible plants have recently been extensively studied to identify their potential and reintegrate them into modern cuisine. More often than not, studies have shown that edible plants, including their leaves, stems, roots, and fruits, often have higher concentrations of nutrients and bioactive compounds compared to cultivated species (Trichopoulou et al., 2000; Fernández-Ruiz et al., 2016).

In the Mediterranean region, edible plants are still a central component of traditional cuisine. Among other countries, Türkiye especially has a tremendously rich biodiversity and a cultural heritage in terms of edible plant consumption (Dogan, 2012). Global efforts have arisen for the protection of such heritage, leading to the Biodiversity for Food and Nutrition Project, led by Brazil, Kenya, Sri Lanka, and Türkiye, and implemented by Biodiversity International with support from the United Nations Environment Program and the Food and Agriculture Organization of the United Nations (Borelli et al., 2020). Within the project, extensive market surveys were conducted with more than 2000 local edible plant gatherers in Türkiye (Tan et al., 2017), and 42 species of edible plant were prioritized (Hunter et al., 2019). In addition, local gatherers and younger generations were educated to recognize and collect local edible plants (Borelli et al., 2020). The scientific community has been contributing to the mission as well by conducting several projects revealing the nutritional and bioactive properties of edible plants (Hacıseferoğulları et al., 2012), including their phenolic compounds.

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The oriental hackberry tree *Celtis tournefortii* Lam. (Cannabaceae, coded as Ct) is a deciduous tree, usually about 5 m tall, that grows in high temperate and tropical regions. The edible fruits of this tree are popular in many countries including Türkiye, Ukraine, Croatia, Greece, Iraq, Iran, and Azerbaijan. The fruiting bodies of Turkish oriental hackberry are popularly known as 'dardağan' or 'doğu çitlembiği' by the local people in Türkiye (Yücedağ and Cemal, 2008; Gecibesler, 2019)

Various parts of *Prunus armeniaca* Lam. (Pa), commonly known as the apricot tree, are used medicinally to treat a wide range of diseases, including respiratory, gynecological, and digestive disorders, and for their antipyretic, antiinflammatory, hepatoprotective, vulnerary, anthelmintic, and anticancer properties (Kitic et al., 2022).

Morus alba Linn (Ma), commonly known as white mulberry, belongs to the family *Moraceae* and is also known as 'dut' in India. *Morus alba* is a moderately sized tree, 3–6 m high. White mulberry is cultivated throughout the world wherever silkworms are raised (Devi et al., 2013).

Over 80 species of the genus *Crocus* L. (Cc) have been identified worldwide (Noroozi et al., 2020). The most common species of this genus is *Crocus sativus*. Saffron, the costliest food colorant and flavor in the world, is derived from the dried stigmas of *C. sativus* and has been used in folk medicine to treat a range of disorders since ancient times (Bhandari, 2015; Yaribeygi et al., 2019; Ghaffari and Roshanravan, 2019; Noroozi et al., 2020;Shakeri et al.,2022).

Chickpea (*Cicer arietinum* L., Ca) is an ancient selfpollinated legume crop believed to have originated in southeastern Türkiye and the adjoining region of Syria. The major goals of chickpea breeding are to increase production either by upgrading the genetic potential of cultivars or by eliminating the effects of diseases, insects, drought, and cold (Singh, 1997).

Gundelia tournefortii L. (Gtr for shoot, Gts for stem) is an important food source and a well-known medicinal plant in eastern Anatolia. The therapeutic effects of medicinal plants are known to be closely related to their antioxidant capacities (Çoruh et al., 2007).

Rheum ribes L. (rhubarb, Rr) belongs to *Polygonaceae,* and its roots and fresh shoots are consumed as vegetables in Turkey. This plant is considered one of the most important pharmaceutical raw materials in the Middle East (Keser et al., 2020).

Eremurus spectabilis M. Bieb (Es) has been extensively investigated both experimentally and theoretically, including on the antioxidant properties of its flavonoids, hydroxycinnamic acid derivatives, hydroxybenzoic acid derivatives, and organic acids (Tegin et al., 2024).

Phenolic compounds have been reported to have positive effects against several disorders, including cardiovascular diseases, certain types of cancer, diabetes, and osteoporosis. These effects are linked to the antioxidant, anti-inflammatory, and antimicrobial activities of these compounds (Zielinski et al., 2014). Current evidence emphasizes that the beneficial effects of phenolic compounds are largely dependent on their bioaccessibility and bioavailability, which are defined as "the amount of identified compounds available for absorption in the gastrointestinal tract and the fraction of such compounds that reach circulation that are available for use", respectively (Lorenzo et al., 2019). However, besides the work of the present author (Ozkan et al., 2022), none of the previous research on local edible plants and fruits focused on the bioaccessibility and bioavailability of phenolic compounds throughout the gastrointestinal system. Therefore, the present study aims to investigate the fate of the phenolic compounds of different parts of nine edible plants, including fruits, corms, seeds, shoots, leaves, and stems, by comparing their total phenolic and flavonoid content (TPC and TFC, respectively) and antioxidant activity throughout the gastrointestinal digestion model.

2. Materials and methods

2.1. Plant materials

The plant material was collected by Dr. Bayram Yurt from the provinces of Bingöl and Malatya during the vegetation period of 2022. The collected plants were identified by Dr. Yakup Yapar using the relevant volumes of the Flora of Turkey (Davis 1967; 1970; 1972; 1975; 1982; 1984). One sample of each plant is kept in the Bingöl University Herbarium. Information about the studied plant species is given in Tables 1 and 2. The Ct, Ma, and Gts samples were provided dried (air dried) whereas all of the other samples were provided in fresh form. Before analysis, the freshly obtained Pa, Cc, Ca, Gtr, Rr, and Es samples were freeze dried.

2.2. Chemicals

For simulated in vitro digestion, α-amylase (EC 3.2.1.1, from human saliva), pancreatin (8 × USP, EC 232.468.9), and bile salt were used. For TPC, TFC, and antioxidant activity, gallic acid, (+) – catechin, Folin–Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazil (DPPH) and neocuproine, methanol (75%), ethanol (96%), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and aluminum chloride hexahydrate ($AlCl_3.6H_2O$), and copper (II) chloride ($CuCl_2$) and ammonium acetate (NH_4Ac) were purchased from Sigma-Aldrich (Steinheim, Germany).

Table 1. Plant species with their common names.

Plant name (Latin)	Parts to be used	Common names	Sample code
Celtis tournefortii Lam.		Oriental hackberry	Ct
Prunus armeniaca L.	Fruit	Apricot	Pa
Morus alba L.		Mulberry	Ma
Crocus cancellatus Herb. subsp. *damascenus* (Herb.) B.Mathew	Corm	Crocus	Cc
Cicer arietinum L.	Seed	Chickpea	Ca
Gundelia tournefortii L. var. *tournefortii*	Shoot	Tumbleweed Tumble thistle Gundelia	Gtr
Rheum ribes L.		Rhubarb	Rr
Gundelia tournefortii L. var. *tournefortii*	Stem	Tumbleweed Tumble thistle Gundelia	Gts
Eremurus spectabilis M.Bieb.	Leaf	Foxtail lily	Es

Table 2. Plant species collection.

Family	Species	Growth period	Locality	Alt. (m)	Date	Collector no.
Cannabaceae	*Celtis tournefortii*	Fruit	Türkiye: Malatya, Darende District, Ilıca village	1100	01.10.2021	B.Yurt09
Iridaceae	*Crocus cancellatus* subsp. *Damascenus*	After flowering	Türkiye: Bingöl: Genç District, Şehit village	1350	15.06.2022	B.Yurt07
Asteraceae	*Gundelia tournefortii* var. *tournefortii*	Before flowering	Türkiye: Bingöl: west of city centre	1125	25.05.2022	B.Yurt05
Polygonaceae	*Rheum ribes*	Flowering	Türkiye: Bingöl: Yelesen village	2000	23.05.2022	B.Yurt04
Xanthorrhoeaceae	*Eremurus spectabilis*	Before flowering	Türkiye: Bingöl: Yelesen village	1900	05.04.2022	B.Yurt01
Asteraceae	*Gundelia tournefortii* var. *tournefortii*	Flowering	Türkiye: Bingöl: west of city centre	1125	05.04.2022	B.Yurt02
Fabaceae	*Cicer arietinum*	Fruit	Türkiye: Bingöl city centre	1150	02.06.2022	B.Yurt06
Rosaceae	*Prunus armeniaca*	Early fruiting period	Türkiye: Malatya, Darende District	1100	20.04.2022	B.Yurt03
Moraceae	*Morus alba*	Fruit	Türkiye: Malatya, Darende District	1100	01.08.2022	B.Yurt08

2.3. Preparation of samples

All samples were ground for 1 min with a coffee grinder (Sinbo, SCM 2934) and stored at room temperature for further analysis. The procedure described by Capanoglu et al. (2008) was performed to extract phenolics from the samples. After weighing 1.00 ± 0.01 g of the powdered samples in three independent groups (n = 3) into 15 mL falcon tubes, 5 mL of 75% methanol solution was added. The mixtures were vortexed for 10 s and left in an ultrasonic bath for 15 min. All samples were centrifuged at 4 °C at 2700 × g (4000 RPM) for 10 min, and the supernatants were transferred to clean tubes. This process was performed again so that the two upper phases obtained made up a volume of 10 mL. The prepared extracts were stored at –20 °C for use in further analyses.

2.4. In vitro gastrointestinal digestion model

To evaluate bioaccessibility, an in vitro gastrointestinal digestion procedure based on a study by Minekus et al. (2014) was conducted. This protocol includes sequentially simulated oral, gastric, and intestinal digestion steps. To simulate oral digestion, 5 g of each sample was mixed with 3.5 mL of salivary juice, 0.5 mL of α-amylase solution (25 μkat/mL), 25 μL of 0.3 M $CaCl_2$, and 0.975 μL of distilled water to a final volume of 10 mL. This mixture was incubated for 2 min at 37 °C in a shaking water bath (Memmert SV 1422, Memmert GmbH & Co. Nürnberg, Germany). To simulate gastric digestion, 7.5 mL of gastric juice, 1.6 mL of pepsin solution (417 μkat/mL), and 5 μL of 0.3 M $CaCl_2$ were added into the oral bolus and the pH

was fixed to 3.0 using 1 M HCl. The total volume of this mixture was adjusted with distilled water to 16 mL and the mixture was incubated for 2 h in a shaking water bath at 37 °C. After the simulated gastric digestion, 5 mL aliquots were collected from each extract. The intestinal digestion was simulated by adding 8.25 mL of intestinal juice, 3.75 mL of pancreatin (13 μkat/mL), 1.875 mL bile (160 M), and 30 μL of $CaCl_2$ (0.3 M) into the remaining gastric chyme and the pH was adjusted 1 M NaOH. Distilled water was used to bring the final total volume to 28 mL. The mixture was incubated for 2 h in a shaking water bath at 37 °C. A blank (the same amount of water instead of samples) was also kept under the same conditions in order to eliminate any interference from the fluids of the simulated digestive system. All samples obtained from the simulated gastric and intestinal digestion phases were centrifuged (Hettich, Tuttlingen, Germany) at 23,000 g and 4 °C for 5 min. Then, the supernatants were stored at –20 °C until further analysis.

2.5. Identification and quantification of polyphenols by HPLC-PDA

The TPCs of the plants were identified using the procedure of Capanoglu et al. (2008). Concisely, samples were passed through 0.45-μm membrane filters before being injected into a high-performance liquid chromatography (HPLC) system with a photodiode array (PDA) detector (Waters 2695, Waters 2996). Supelcosil LC-18 (25 cm × 4.60 mm, 5 m column, Sigma-Aldrich) was used for the stationary phase.

The mobile phases were TFA (trifluoroacetic acid)/MQ (deionized water) (1 mL/L; eluent A) and TFA/acetonitrile (1 mL/L; eluent B); these were used for the spectral measurements at 280, 312, and 360 nm with an injection volume of 10 mL and a flow rate of 1 mL/min.

A linear gradient was used as follows: At 0 min, 95% solvent A and 5% solvent B; at 45 min, 65% solvent A and 35% solvent B; at 47 min, 25% solvent A and 75% solvent B; and at 54 min. At the end of the 54 min, linear gradients returned to initial conditions at 0 min. Phenolic acids were identified and quantified using their authentic standards (gallic acid, protocatechuic acid, syringic acid, chlorogenic acid, *p*-coumaric acid, 4-hydroxybenzoic acid, epicatechin, rutin, and quercetin). All analyses were performed in triplicate, and the results were expressed as mg/100 g of sample.

2.6. Determination of total phenolics and antioxidant activity

TPC was analyzed using a Folin–Ciocalteu (FC) reagent as per Singleton and Rossi (1965). A 15-μL aliquot of extract and 112.5 μL of Folin–Ciocalteu reagent were mixed in a well plate and kept for 5 min at room temperature. Then, 112.5 μL of 6% Na_2CO_3 solution was added to the mixture, which was then incubated in darkness for 1 h at room temperature. After the incubation period, the absorbance values of the mixtures were measured with a spectrophotometer at 765 nm. To calculate the TPC of the analyzed samples, a calibration curve was used ($R^2 = 0.9992$, $y = 3.6822x - 0.0147$, x: concentration, y: absorbance). The results were obtained as mg gallic acid equivalents (GAE) per 100 g sample.

The TFC assay was conducted based on a method applied by Dewanto et al. (2002). The results were stated as mg catechin equivalents (CE) per 100 g sample. The principle of the procedure is based on the adhesion of Al to the cyclic structure and causing an alteration in the mixture color with the effect of NaOH. A 75-μL aliquot of 5% $NaNO_2$ was added to 0.25 mL of extract before incubating for 6 min at room temperature. Then, 150 μL of 10% $AlCl_3.6H_2O$ was added, and 5 min later, 500 μL of 1 M NaOH was also added to the mixture. The final volume of the mixture was adjusted to 2.5 mL using 1525 μL of distilled water. The absorbances of the samples were determined with a spectrophotometer at 510 nm. A calibration curve was used to obtain the TFC amounts of the analyzed samples ($R^2 = 0.993$, $y = 2.15x - 0.0318$, x: concentration, y: absorbance).

The total antioxidant activity of the samples was determined by DPPH and cupric-reducing antioxidant capacity (CUPRAC) assays (Apak et al., 2004; Hara et al., 2018). For all protocols, the results were given as mg Trolox equivalents (TE) per 100 g of sample. A 10-μL aliquot of extract and 200 μL of DPPH reagent (dissolved in 0.1 mM methanol) were mixed in the DPPH method and left for 30 min to incubate in a dark environment at room temperature after 10 s of shaking. At the end of the incubation period, absorbance values were measured using the spectrophotometer at 517 nm. A calibration curve was used to find the total antioxidant capacity (TAC) of the samples ($R^2 = 0.990$, $y = 4.0161x + 0.0792$, x: concentration, y: absorbance). To apply the CUPRAC method, 70 μL of 10 mM copper (II) chloride, 70 μL of 7.5 mM neocuproine, 70 μL of 1 mM ammonium acetate (pH 7.0), and 70 μL of distilled water were mixed with the 7 μL of extract. After shaking for 10 s at room temperature, the mixture was incubated for 30 min in the darkness. When the incubation period was over, absorbance was measured using a spectrophotometer at 450 nm. The TAC of the analyzed samples was determined using a calibration curve ($R^2 = 0.991$, $y = 2.4117x - 0.0164$, x: concentration, y: absorbance).

2.7. Statistical analysis

All experiments were done by performing three parallel measurements on each sample extract prepared in three replicates. The results were stated as mean ± standard deviation. Statistical analysis was carried out with SPSS v.28.0 (SPSS Inc.) and the data were compared using one-way analysis of variance followed by a Tukey post-hoc test ($p < 0.05$).

3. Results

3.1. Spectrophotometric analysis

The present study focused on the in vitro bioaccessibility of the phenolic compounds of nine different edible plants and fruits collected from Bingöl, Türkiye. Table 3 reports the changes in TPC and TFC of samples during in vitro digestion. Both TPC and TFC differed significantly ($p < 0.05$) between undigested (UD), gastric digested (GD), and intestinal digested (ID) samples. Overall, the UD stem samples, namely Es (48.98 ± 5.16 mg GAE/100 g) and Gts (838.3 ± 66.7 mg GAE/100 g), had the lowest TPC values with moderate TFC values.

Simulated digestion had varying effects on the plant samples. For TPC, all fruit and stem samples, except Pa, had remarkably increased TPC values (between ~0.3–7-fold) following GD ($p < 0.05$). However, all samples had lower TPC values after ID compared to the GD samples. For TFC, the values of the fruit and stem samples, except Ct, Ma, and Cc, significantly decreased after GD compared to the UD samples. It should be noted that despite the decrease in TFC after GD compared to UD, all samples showed increased TFC values after ID compared to GD, which differs from the TPC results.

Monitoring only TPC and TFC values during gastrointestinal digestion would lead to limited information, since the bioactivity of distinct types of phenolic acids and flavonoids are different from each other. Thus, there is also a need for antioxidant activity measurements. In this study, both DPPH and CUPRAC antioxidant activity assays were used (Table 4).

Overall, the samples had significantly different levels of antioxidant activities from each other, as well as at different stages of gastrointestinal digestion ($p < 0.05$). The DPPH results showed that all samples had higher antioxidant activity after GD compared to the UD samples. Especially the Ct, Cc, and Gtr samples had a remarkable increase in bioactivity of 70-fold, ~11-fold, and ~4-fold, respectively. However, the CUPRAC assay results had a completely different trend; all samples except Pa, Ma, and Rr had increased antioxidant activity following GD, which then decreased after ID.

Table 3. Changes in the TPC and TFC of the samples during in vitro digestion.

Sample	TPC (mg GAE/100 g)			TFC (mg CE/100 g)		
	UD	GD	ID	UD	GD	ID
Ct	198.3 ± 18.5^{dC}	1602 ± 130^{bA}	938.9 ± 143.6^{bB}	74.0 ± 8.99^{fC}	593.7 ± 169.1^{aB}	1738 ± 122.1^{aA}
Pa	1026 ± 16^{4a}A	101.3 ± 17.9^{fB}	86.63 ± 11.20^{fB}	712.0 ± 71.7^{aA}	42.30 ± 5.16^{dB}	62.31 ± 2.01^{dB}
Ca	97.88 ± 11.37^{eC}	274.2 ± 33.87^{efA}	238.7 ± 17.89^{eB}	134.8 ± 16.19^{efA}	67.72 ± 8.06^{dB}	64.97 ± 5.16^{dB}
Ma	366.8 ± 44.47^{cC}	1315 ± 86.3^{cA}	583.9 ± 24.95^{cB}	157.9 ± 15.61^{deB}	190.2 ± 6.46^{cA}	200.2 ± 19.74^{dA}
Cc	146.0 ± 9.65^{deC}	479.6 ± 58.5^{dA}	387.0 ± 13.04^{dB}	176.9 ± 22.7^{deB}	175.7 ± 17.65^{cB}	462.2 ± 19.09^{cA}
Gtr	838.3 ± 66.7^{bC}	2216 ± 121^{aA}	1517 ± 95^{aB}	594.37 ± 32.40^{bB}	468.3 ± 21.36^{bC}	729.8 ± 16.61^{bA}
Rr	305.5 ± 34.7^{cA}	305.2 ± 31.65^{deA}	116.8 ± 8.04^{fB}	383.2 ± 31.35^{cA}	54.47 ± 2.14^{dB}	80.33 ± 1.82^{eB}
Es	48.98 ± 5.16^{eB}	148.6 ± 18.09^{efA}	47.74 ± 5.42^{fB}	171.7 ± 10.09^{deA}	33.75 ± 1.38^{dC}	60.20 ± 1.93^{dB}
Gts	97.93 ± 8.49^{eB}	123.4 ± 6.95^{fA}	85.07 ± 15.26^{fB}	217.2 ± 28.10^{dA}	43.78 ± 3.87^{dB}	67.00 ± 0.61^{dB}

All values are mean ± standard deviation. Different lower-case letters in columns or upper-case letters in rows represent statistically significant differences ($p < 0.05$). UD = undigested; GD = gastric digestion; ID = intestinal digestion.

Table 4. Changes in the antioxidant activity of the samples during in vitro digestion.

Sample	DPPH (mg TE/100 g)			CUPRAC (mg TE/100 g)		
	UD	GD	ID	UD	GD	ID
Ct	64.65 ± 4.73^{gC}	4531 ± 436^{aA}	3020 ± 116^{aB}	66.92 ± 4.25^{eC}	1492 ± 232^{bB}	3766 ± 80^{aA}
Pa	641.3 ± 63.3^{aA}	191.6 ± 6.32^{dB}	199.3 ± 14.8^{fB}	1306 ± 8.9^{cA}	174.6 ± 32.7^{dC}	216.2 ± 10.0^{fB}
Ca	129.9 ± 10.1^{fC}	339.5 ± 17.5^{cdA}	278.0 ± 22.1^{efB}	327.3 ± 29.0^{dB}	169.8 ± 13.4^{dC}	358.1 ± 12.1^{eA}
Ma	143.7 ± 6.22^{efC}	602.4 ± 50.2^{cA}	526.2 ± 26.0^{dB}	3198 ± 89^{bA}	1665 ± 196^{bB}	1010 ± 45.5^{dC}
Cc	$183.3 \pm 39.8d^{eC}$	2083 ± 54.8^{bA}	1554 ± 44^{cB}	44.85 ± 4.69^{eC}	359.5 ± 45.3^{cdB}	1821.8 ± 65.5^{cA}
Gtr	537.5 ± 28.6^{bC}	2225 ± 106^{bA}	1903 ± 76^{bB}	1286 ± 8.77^{cC}	2984 ± 219^{aA}	2762.9 ± 155.6^{bB}
Rr	351.4 ± 27.6^{cB}	469.3 ± 46.2^{cdA}	361.4 ± 36.5^{eB}	4485 ± 57.9^{aA}	528.1 ± 34.8^{cB}	412.1 ± 17.2^{eC}
Es	166.2 ± 7.49^{defB}	356.0 ± 12.1^{cdA}	355.6 ± 36.7^{eA}	31.05 ± 3.96^{eC}	426.1 ± 47.1^{cdA}	312.7 ± 4.9^{efB}
Gts	210.6 ± 20.9^{dC}	350.3 ± 13.9^{cdA}	257.4 ± 23.7^{efB}	368.8 ± 60.1^{dA}	199.1 ± 13.8^{dB}	350.1 ± 9.5^{eA}

All values are mean ± standard deviation. Different lower-case letters in columns or upper-case letters in rows represent statistically significant differences ($p < 0.05$). UD = undigested; GD = gastric digestion; ID = intestinal digestion.

3.2. Chromatographic analysis

The TPC values of the UD samples as determined by the FC method correlated with the chromatographic results (Table 5). Also in accordance with the FC method, Pa showed the highest TPC (677.48 ± 113.6 mg/100 g), and the lowest value was found in Es (1.58 ± 0.44 mg/100 g). However, significantly lower TPC values were obtained for all samples based on the HPLC-PDA, and, inconsistent with the FC results, most samples had decreased TPC values following both GD and ID. On the other hand, both significant increases and decreases were observed in each phenolic content during in vitro gastrointestinal digestion compared to the UD samples ($p < 0.05$).

4. Discussion

Among all the undigested samples, the lowest TPC values were determined for the stem samples (Es and Gts). This can be attributed to the fact that phenolic compounds mostly

Table 5. Changes in the individual phenolic compounds of the samples during simulated in vitro gastrointestinal digestion.

Sample	*Phenolic compound*	*UD (mg/100g)*	*GD (mg/100g)*	*ID (mg/100g)*
Ct	Gallic acid	2.75 ± 0.00^{b}	1.95 ± 0.12^{c}	8.36 ± 0.05^{a}
	p-coumaric acid	2.98 ± 0.00^{a}	0.51 ± 0.05^{c}	0.76 ± 0.19^{b}
	Rutin	2.93 ± 0.00	nd	nd
	Quercetin	1.01 ± 0.00	nd	nd
Pa	Gallic Acid	0.55 ± 0.11^{b}	0.59 ± 0.17^{b}	1.41 ± 0.28^{a}
	Protocatechuic acid	1.17 ± 0.00	nd	nd
	Syringic acid	16.55 ± 0.49^{a}	3.69 ± 0.82^{b}	2.07 ± 0.35^{c}
	Chlorogenic acid	488.25 ± 84.13^{a}	12.51 ± 1.81^{b}	0.43 ± 0.00^{b}
	p-coumaric acid	8.60 ± 0.08	0.33 ± 0.00	nd
	Rutin	160.70 ± 28.15	3.42 ± 0.20	nd
	Quercetin	1.66 ± 0.43	nd	nd
Ca	Gallic acid	0.45 ± 0.02^{c}	15.85 ± 2.17^{a}	6.28 ± 1.05^{b}
	4-hydroxybenzoic acid	0.79 ± 0.10^{b}	2.47 ± 0.25^{a}	0.31 ± 0.05^{c}
	Syringic acid	4.19 ± 0.41^{b}	6.85 ± 0.17^{a}	6.57 ± 1.25^{a}
Ma	Syringic acid	1.92 ± 0.70^{c}	8.00 ± 1.80^{b}	10.88 ± 0.42^{a}
	Chlorogenic acid	8.60 ± 0.13	nd	nd
	p-coumaric acid	0.52 ± 0.05^{b}	0.56 ± 0.02^{b}	1.09 ± 0.00^{a}
	Rutin	14.17 ± 1.44^{a}	8.57 ± 0.79^{b}	2.27 ± 0.19^{c}
Cc	Gallic acid	0.56 ± 0.06^{b}	0.70 ± 0.24^{b}	1.12 ± 0.00^{a}
	Protocatechuic acid	5.89 ± 0.34	2.27 ± 0.00	nd
	Syringic acid	3.67 ± 0.40^{a}	1.76 ± 0.00^{b}	1.30 ± 0.00^{b}
	Quercetin	0.46 ± 0.03	nd	nd
Gtr	Gallic acid	1.06 ± 0.08^{c}	1.42 ± 0.20^{b}	3.37 ± 0.25^{a}
	Syringic acid	8.53 ± 0.26^{a}	3.21 ± 0.01^{c}	4.00 ± 0.18^{b}
	Chlorogenic acid	490.38 ± 29.91	13.45 ± 3.81	nd
	p-coumaric acid	1.23 ± 0.20	1.12 ± 0.02	nd
Rr	Gallic acid	0.45 ± 0.08^{c}	1.59 ± 0.44^{b}	3.11 ± 0.12^{a}
	Syringic acid	2.49 ± 0.22^{b}	3.21 ± 0.01^{b}	4.35 ± 0.68^{a}
	Epicatechin	9.46 ± 0.63	4.05 ± 0.04	nd
	p-coumaric acid	0.50 ± 0.14	0.30 ± 0.09	nd
Es	Gallic acid	0.63 ± 0.18^{b}	1.03 ± 0.02^{b}	2.69 ± 0.45^{a}
	Chlorogenic acid	0.95 ± 0.26	0.95 ± 0.14	nd
Gts	Chlorogenic acid	2.80 ± 0.64^{b}	8.57 ± 0.21^{a}	0.80 ± 0.01^{c}
	Rutin	5.89 ± 0.24	1.28 ± 0.19	nd
	Quercetin	0.62 ± 0.06	nd	nd

Different lower-case letters in the rows show statistically significant differences ($p < 0.05$). UD = undigested; GD = gastric digestion; ID = intestinal digestion; nd = not detected.

accumulate in leaves due to their role in photosynthesis and defense against physiological stress (Chowdhary et al., 2022). Also, the robust structure of stem tissue might have resulted in poor extraction of these compounds. After GD, most plants showed a significant increase in TPC values ($p < 0.05$), while a decrease was observed for all samples after ID compared to the gastric phase. This type of initial increase has been reported elsewhere as linked to the release of bound phenolics due to acidic digestion (Kamiloglu et al., 2022). Moreover, the later reduction in phenolic compounds may be linked to these compounds being prone to oxidation, polymerization, transformation, and complex formation with metal ions and proteins (Velderrain-Rodríguez et al., 2014).

Similar to the TPC result, the highest TFC value was determined in Pa as 712.0 ± 71.7 mg CE/100g. The TFC of all samples except Ct and Ma decreased after the gastric phase. In parallel with these findings, previous studies determined a reduction in TFC values after GD, which may be associated with pH conditions. At an acidic pH, flavonoid-protease complexes may form as a result of the interaction of the flavonoids with protease. It has been reported that depending on the gastric or intestinal pH circumstances, the strength of binding between catechin and digestive enzymes can vary. The content of dissolved flavonoids in low-pH solutions may be reduced by the attachment of flavonoids to pepsin (Su et al., 2018). However, all samples except Pa had a TFC increase following ID, in contrast with the TPC results. Qin et al. (2018) reported that several phenolic compounds are differently affected by intestinal enzymes that can release nonextractible phenolic compounds based on their structural properties, and these compounds can still be released during ID.

Following ID, the second phase of digestion, the antioxidant activity of the Ct, Cc, and Gtr samples, which increased after the GD phase according to the CUPRAC assay, had somewhat decreased values, as analyzed by the DPPH assay. As discussed before, the results from both antioxidant activity assays did not complement the TPC values throughout digestion; this may be due to the unselective nature of the FC assay that might have led to misinterpretation of the phenolic compound content (Kamiloglu et al., 2017). There was a better correlation antioxidant activity and TPC according to the FC assay, compared to the TPC obtained from the HPLC-PDA. In addition, the results of the two antioxidant assays differ from each other. It has been reported that the CUPRAC assay includes chemicals soluble in both organic and aqueous solvents, whereas DPPH is mainly soluble in organic solvents, which limits the determination of the antioxidant activity of hydrophilic compounds (Özyürek et al., 2011).

Although the FC assay is currently one of the most widely used, efficient, and simplest methods to evaluate phenolic contents of foods, it has some drawbacks because of low specificity. According to reports, many nonphenolic compounds that are chemically similar, such as bioactive peptides, may interfere with the assay results; the FC reagent may be decreased by such compounds, so it is nonspecific to phenolics. Therefore, in the present study, HPLC-PDA was also applied to determine the individual phenolic contents of the undigested and digested samples. In comparison with the FC method, TPC values were found to be significantly lower from HPLC-PDA. This may be related to the FC reagent not being specific to phenolics and possibly being affected by reducing sugars and organic acids (Batista et al., 2017). Different phenolic compounds were detected in the samples. Gallic acid, syringic acid, chlorogenic acid, and *p*-coumaric acid were mostly found in the samples. Gallic acid increased during both phases of gastrointestinal digestion, except for the samples Ct and Ca. The rise in gallic acid level may be directly linked to the gallotannin hydrolysis within the plants. Moreover, the bioaccessibility of the phenolic compounds may increase significantly when the acidic environment of the stomach changes to the slightly alkaline condition of the intestinal phase, suggesting that the release of compounds from the plant matrix is allowed by the intestinal conditions (de Paulo Farias et al., 2021). Following the gastrointestinal digestion, chlorogenic acid, which was at a high level prior to digestion, drastically decreased in the Pa, Ma, and Gtr samples, but increased in the Gts sample. Pa (11.5%), Ma (8.02%), and Gtr (~24.7%) are rich in fiber content (Ali et al., 2015; Mehmood Abbasi et al., 2016; Esbati et al., 2021). The bioaccessibility of phenolics may be limited by interaction with fiber that is released during digestion because when bioactive compounds interact with fiber in gastrointestinal digestion fluids, they become minimally extractable and soluble (Lucas-González et al., 2018). Therefore, the complexity of the food matrix, the formation of phenolic metabolites, and the environmental conditions may affect the individual phenolics differently during gastrointestinal digestion.

5. Conclusions

Edible plants and fruits have been commonly consumed as an antioxidant source linked to several disease treatments. This study aimed to examine the TPC, TFC, and total antioxidant capacities of some plants as well as the bioaccessibility of phenolics in plants using a simulated in vitro digestion procedure. It can be deduced that regular intake of the edible plants analyzed in this study may have positive effects on health due to their high antioxidant activity. The highest TPC and TFC values among all undigested samples were found in Pa (1026 ± 164 mg

GAE/100 g and 712.0 ± 71.7 mg CE/100 g, respectively). The lowest TPC (48.98 ± 5.16 mg GAE/100g) was detected in undigested Es, and Ct had the lowest TFC (74.0 ± 8.99 mg CE/100 g). Most of the samples had increased TPC values after in vitro digestion, whereas most of their TFC values diminished. Moreover, when correlated with the TPC, the antioxidant activity of most samples decreased according to the CUPRAC assay, whereas there was an increase compared to the DPPH method. Considering these results, phenolic compound levels and their antioxidant capacities both varied depending on the interaction of different types of enzymes released during digestion with a complex food matrix and the conditions of the digestive environment (low or alkaline pH). As a next stage, conducting in vitro and Caco-2 cell culture-based assays together to evaluate the fate of plant phenolic contents in the human digestive system may useful to assess bioaccessibility.

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